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BIOCHEMICAL FUEL CELLS

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FIRST QUARTERLY REPORT

22 MARCH 1963 THROUGH 31 JULY 1963

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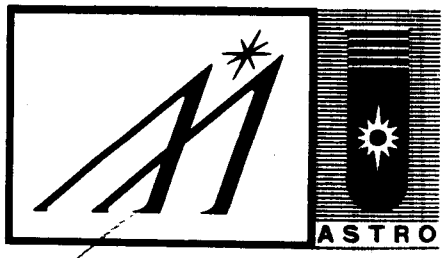

THE MARQUARDT CORPORATION

THE Marquardt CORPORATION VAN NUYS, CALIFORNIA

STUDY OF BIOCHEMICAL FUEL CELLS

First Quarterly Progress Report
22 March 1963 through 31 July 1963
Contract NASw-654

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Prepared by:

George E. Ellis
Principal Investigator

Edward E. Sweeney
Microbiologist

Checked by:

J. G. Bitterly
Principal Research Scientist

Approved by:

A. N. Thomas
Chief Research Scientist
Advanced Projects

THE MARQUARDT CORPORATION
16555 Saticoy Street
Van Nuys, California

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DISCLAIMER

This report has not been approved by the National Aeronautics and Space Administration, and the conclusions reached herein do not necessarily reflect those of the contracting agency.

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ABSTRACT

The purpose of the investigations described in this report was to conduct empirical studies on biochemical fuel cells for degrading human wastes and producing electrical energy therefrom. Specifically, the study includes the attachment of organisms to electrode materials, electrode pretreatment and configuration, the selection of suitable organisms and separator materials, selection of electrolytes and additives, structural materials and control devices, and storage and performance characteristics.

During the First Quarter, investigations have indicated that either human urine or human feces (non-sterile), or mixtures of urine and feces, can be employed as biofuels in a biochemical battery. Open-circuit potentials as high as 0.950 volts, and short-circuit currents as high as 1.5 milliamperes per square centimeter, have been attained thus far, from cells employing a bio-anode and a non-biological (air) cathode. Additions of chemicals or extraneous micro-organisms have been made to the human waste in some cases, to test the activities of non-indigenous organisms and to provide nutrient and buffer pH. Ideally, it may be possible to forego these additions.

A limited number of experiments have been made with bio-cathodes employing sulfate or nitrate reducers, but they were not more effective than the non-biological cathode, and further studies are being held in abeyance in order to place emphasis on the bio-anode reactions.

Studies of pretreatment of the human waste have been conducted, in an effort to maximize the electrical power output. The literature describing sewage treatment is being consulted to aid this program. The variables being investigated have thus far been confined to temperature, time, vessel material, concentration, atmospheric conditions, and pH. Results have not yet been conclusive.

A single preculturing experiment was conducted, but the results were unsatisfactory.

The electrode materials used thus far have been limited to platinized platinum foil or screen. Separator materials have been limited to agar, and a single sample each of cellulose acetate, anion exchange membrane, and cation exchange membrane; the cellulose acetate membrane seems to be satisfactory and simple to use.

Polarization and power curves have only recently been obtained. Power curves obtained from the polarization curves have indicated a peak power of approximately 1.45 microwatt per square centimeter. The duration of each polarization curve was six hours.

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A literature survey and laboratory test results have indicated that the materials used in the present experimental systems are compatible with the micro-organisms.

The items of research being emphasized at present are designed to improve the homogenization techniques, and increase the reproducibility of the electrical output of the biochemical systems.

Apparatus and equipment construction and installation includes static and continuous flow systems, reference electrodes and salt bridges, impedance bridge, 50-channel digital voltmeter, and X-Y polarization curve plotter.

A supply of human feces is being collected from volunteers on a simulated astronaut's diet.

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STUDY OF BIOCHEMICAL FUEL CELLS

Introduction

This report covers the progress attained during the First Quarter, inclusive to 31 July 1963, for the Study of Biochemical Fuel Cells, Contract No. NASw-654. The purpose of this program is to conduct empirical studies on biochemical fuel cells for producing electrical energy through degradation of human waste.

I. Bio-Anode Organisms

The selection of the appropriate bio-anode organisms for use with human waste is recognized to be within the area of research conducted by one of the companion firms in this program. However, pending selection of the optimum organisms, some evaluations were made early in this program using Desulfovibrio desulfuricans, Escherichia coli, and more recently, on the utilization of those micro-organisms indigenous to human waste.

Detailed descriptions of the experiments performed to date are presented in Appendix A.

It has been found that micro-organisms indigenous to human feces or urine, or the addition of Escherichia coli to human waste, are effective in promoting reactions which are capable of generating electrical power when used as a bio-anode. The choices of Escherichia coli and the indigenous micro-organisms have been partially substantiated by a literature survey conducted by one of the companion firms in this program.

Some of the studies have been made with sterile media added to the bio-fuel, to provide nutrient and buffer pH. The addition of extraneous material to the system is not desirable in a space application, but was done experimentally in order to optimize power output and prolong the life of the fuel cell. Most recently (i.e., for the past month), no extraneous fuels, nutrient, or micro-organisms have been added to the system.

II. Bio-Cathode Organisms

Some studies were made employing micro-organisms at the cathode as well as at the anode; for example, see Experiments 2, 3, 4, 13, 15, 16, 17, and 18, described in Appendix A. However, the study of biochemical cathodes, though recognized in the subject program, are considered to be less important than the study of the biochemical anodes, and have been discontinued for the present. In the limited studies that were conducted, it was found that the combined biochemical anode and biochemical cathode systems produced no greater electrical output than the bio-anode and non-biological (air) cathodes.

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III. Fuel Composition

An investigation has been conducted to determine the optimum concentration of feces and/or urine to be used as a fuel-anolyte.

The reproducibility of these tests has been unsatisfactory and will be discussed in detail in Section X of this report.

Within the limits of reproducibility of these data, it is apparent that either pure urine or mixtures of feces-urine (both non-sterile) in the composition range of 1 percent to 30 percent by weight feces are effective fuels. The addition of micro-organisms or enzymes (e.g., Escherichia coli, Pseudomonas denitrificans, cellulase) has not increased the power output significantly.

IV. Pretreatment of Waste

Some studies have been conducted on the effects of pretreatment of waste to increase its electrochemical activity, by obtaining different reactions and greater potentials, as well as faster reactions and increased current density. The possibility remains that no pretreatment will be desirable because of the attendant loss of energy. Pretreatment experiments are described in detail in Appendix B.

The pretreatment of human waste has included investigations of the relatively simple variables of temperature (23° to 42°C), time (1 to 9 days), vessel material (glass or type 304 stainless steel), concentration of human waste, atmospheric conditions (aerobic or anaerobic), and pH control. The following general results have been obtained:

A. The effects of temperature have not been consistent

B. There has been a general increase in electrical output with time for the first 24 to 36 hours, followed by a slow decrease to a pseudo-plateau after an additional 1 to 7 days

C. Possible inhibitory effects of the stainless steel are being evaluated, and if such effects are found, various materials (Teflon, nickel, porcelain, etc.,) will be evaluated as possible liners for the metal storage vessels.

D. The composition of biofuel currently being used is approximately the same as that of normal elimination -- i.e., 30 grams of feces to 100 milliliter of urine.

E. Anaerobic conditions have been better than aerobic

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F. pH control (below 7.0) has been detrimental. The pH is not controlled at the present time and occasionally rises as high as 8.8 from an initial pH of 6.1 to 7.1.

V. Transfer (Preculturing) Methods

A single experiment has been conducted in an attempt to maintain electrochemical activity of the human waste at a peak level, by transferring a portion of the waste at its peak power output (e.g., 24 hours old) to fresh waste; the fresh waste constituted 90 percent of the mixture by weight. The fresh material contained 30 grams of feces and 100 milliliters of urine (both non-sterile). The original attempt was neither harmful nor beneficial, and the mixture of old and new material behaved essentially the same as new material. Details of this experiment are presented in Table VIII of the Appendix.

Further studies will be made, possibly by transferring activated sludge from sewage.

VI. Electrodes and Electrode Reactions

The electrodes used in the experiments described in this report have been either platinized platinum foil, in the static glass H-cells, or platinized platinum screen, in the plastic continuous flow systems. Other electrode materials have not been used, and will not be used until the reproducibility of the present systems can be improved to a practical degree. At that time, more economically attractive electrode materials will be studied.

Some limited studies of polarization and overpotentials, limiting current densities, and other phenomena of electrode kinetics are being initiated, commensurate with the investigations of the co-contractors.

Reference electrodes (saturated calomel) and Luggin capillary salt bridges have recently been installed in all experimental systems. All future values of electromotive potentials will be based upon the reference electrodes.

VII. Separator Materials

No extensive studies have been made of separator materials, and such studies will not be made until a practical degree of reproducibility of electrical power output can be attained in the present experimental systems. Studies have thus far been limited to one anion exchange membrane, one cation exchange membrane, one type of cellulose acetate, and agar plugs. The cellulose acetate separators thus far seem to be reasonably satisfactory, based upon low electrolytic resistance and low contamination rate of anode and cathode chambers, either by micro-organisms or chemical substances.

VIII. Polarization and Power curves

Some typical polarization and power curves are illustrated in Figures 2 and 3. The polarization curves (voltage vs. current) were plotted with an X-Y plotter (Moseley Autograf), modified in this laboratory to produce a decreasing resistance in the external circuit of the cell of approximately 33,000 ohms per hour (i.e., from 200,000 ohms to short-circuit in approximately 6 hours). After obtaining the potential reading at open-circuit, the initial reading under load was at an external resistance of 200,000 ohms. At short-circuit, the resistance in the external circuit was less than 1 ohm. The total time of 6 hours to obtain the polarization curves was chosen, in order to provide as nearly equilibrium conditions as feasible in a changing system, and at the same time, be commensurate with a day's operation in the laboratory.

The power curves were obtained by cross-plotting the potential vs. current data from the polarization curves.

IX. Materials Evaluation and Testing

The possibility of problems regarding the compatibility of materials used in the various experimental systems has been recognized, and a continuing effort is being made to eliminate materials which may exert inhibitory effects on the electrical power characteristics of the biofuel cell, either by their deleterious effects on the micro-organisms or by physically poisoning the electrodes. Answers to these questions are being sought in the literature, by means of tests conducted in the laboratory, and by requesting the co-contractors and all other personnel associated with this program to forward any pertinent information to this laboratory. The results thus far have been encouraging, and there does not seem to be any need for major revisions in the materials currently being used. Personnel from the NASA office have quoted references to investigations which have demonstrated a poisoning effect caused by silicone grease deposited on electrodes; no such stopcock grease is currently being used.

A literature survey has indicated some toxic effects of metals and plastics upon bacteria. It was found that copper, zinc, brass, and butyl rubber were toxic; while tin, lead, aluminum, stainless steel, epoxy resin, polyethylene, silicone rubber, and vinyl were not.* This survey is being continued.

A pretreatment vessel has been constructed of type 304 stainless steel because it is widely used for general chemical plant equipment, is easy to fabricate, and is low in cost. However, the data have indicated a possible

*Rosenwalt, A. J., et al., Applied Microbiology, 10 (1962), pp. 345-347.

deleterious effect of the metal, compared to glass, as evidenced by consistently lower electrical output (up to 0.3 volts less open-circuit potential and up to 0.78 milliamperes per square centimeter less in short-circuit current).

If deleterious effects are experienced, appropriate leaching, cleaning, or surface coating methods will be sought to remove them.

X. Reproducibility; Statistical Analysis

Some limited number of reproducibility tests have been conducted in H-cells containing 30 grams of non-sterile human feces and 100 milliliters of non-sterile human urine (approximately the ratio in which human waste is eliminated), and it was found that the open-circuit potential varies as much as 70 percent and the short-circuit current 700 percent. In a continuous flow system reproducibility test using plastic cells, the anolyte and catholyte reservoirs were common to both cells; everything else was duplicated in both cells (electrodes, structural material, etc.), except the membranes. The open-circuit potentials in these two cells varied as much as 95 percent, (55 percent at the time of starting the test). The anolyte was 25 percent non-sterile human feces; 25 percent non-sterile human urine; and 50 percent sterile sulfate medium. These variations may have been caused by incomplete homogenization; the mixture was prepared in a blender while homogenizing for 15 minutes. Other methods of homogenizing will be attempted followed by filtration.

The Reproducibility tests will be continued during the Second Quarter.

XI. Miscellaneous Studies

In a single experiment to determine the amount of time required to possibly coat or poison the electrodes in the biofuel cells, it was found that a clean pair of electrodes functions about the same as a pair that has been in a cell for twelve days. The decrease in electrical output with time that has been experienced is therefore not caused by continued coating or poisoning of the electrodes. More studies are being made.

Conflicting data regarding the effect of temperature on electrical output of the biofuel cells have been observed. In one set of experiments, the open-circuit potential was less by 0.25 volts after storage at 40°C than after storage at room temperature (23°C); while in another, the opposite was true. All readings of electrochemical properties were made at 23°C to remove temperature effects on the electrochemical properties. The differences in temperature mentioned above, refer to the period of time between readings.

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XII Apparatus and Equipment

The static system is illustrated in Figure 4. The figure illustrates a glass H-cell, consisting of tubing about 7-3/4 inches long by 1-1/8 inch I.D. and has a horizontal distance of 2-1/2 inches between vertical tubes, and a course-fritted glass disc constructed in place. The electrodes are platinized platinum foil, with 1 square inch area on each side, approximately 3-1/2 inches apart. An agar plug pulled into the fritted glass disc by vacuum forms the electrolyte separator. Also illustrated are gas bubblers for bubbling gaseous helium and oxygen (or air) over the anode and cathode, respectively. A Luggin capillary (salt bridge) is shown, to provide measurements of potentials of one electrode (the anode) versus a reference electrode (saturated calomel).

Another type of static cell used in screening experiments is illustrated in Figure 5. This cell is similar to that shown in Figure 4, with the exception that the two halves of the cell are joined with O-ring joints, which are held together with a clamp. The O-ring joints permit the evaluation of separators other than agar. A cellulose acetate separator is illustrated; however, ion exchange or dialysis membranes may also be used. Figure 5 illustrates a cell with a mixture of human feces-urine at the anode and a non-biological (oxygen) cathode.

Many static cells may be used in screening experiments, as illustrated in Figure 6. The rack will accommodate up to 18 cells, so that comparisons of several separator and electrode materials may be conducted simultaneously. The digital printout box in the background will accommodate up to 50 cells, reading voltage or current at prearranged time intervals.

At the top of the rack in Figure 6, another type of glass cell may be observed. It is 4-1/4 inches long, and is contructed of 2-inch O.D. glass tubing. This cell provides larger electrode surface, and the electrodes can be placed closer together (within one-half inch of each other) than in the glass H-cells previously described.

At the lower left of Figure 6, an oxygen meter (Beckman Model OM-1) may be observed; the electrode is in the right arm of the second H-cell (measured from the left). This electrode measures the amount of oxygen in the solution, and is used to establish aerobic or anaerobic conditions required in the cells.

A continuous flow system is illustrated in Figure 7. Two plastic cells are illustrated, connected so that the anolyte and catholyte flasks are common to both. The 5-neck flasks provide for gas bubblers, gas release tube, pH adjustment, nutrient addition, withdrawal of media to be pumped through the cells and returned to the 5-neck reservoirs, and stirring by means of magnetic

stirrers. The cells are constructed of acrylic plastic (Lucite), and the tubing of Tygon. The pumps are located in the center of the photograph. The cells may be clamped-off from each other when taking electrical measurements. In the foreground is a voltmeter, an ammeter, and a decade resistance box.

A dismantled plastic cell is illustrated in Figure 8. From the left in the photograph, are shown a solid end plate, a screen electrode with water repellent and chemically resistant paint to prevent leakage, two electrode reservoirs (one for anolyte and one for catholyte), a second screen electrode, and a second end plate, respectively. A separator, such as a cellulose acetate or ion exchange membrane (not shown, would be placed between the electrolyte reservoirs. The tubes on opposite sides of the reservoirs provide flow into and out of the cell, while threaded nylon rods are passed through the holes at each corner to hold the cells in place. The plastic cell is 3 inches square and has electrodes 1-5/16 inches apart, with an exposed diameter of 2-1/8 inches. It is constructed of 80-mesh platinized platinum screen.

An impedance bridge (Universal Model 291) is illustrated in Figure 9. By means of this bridge, both resistance and capacitance can be balanced when measured electrolytic conductivities and resistances of the many electrolytic solutions and separators currently studied in this program.

A 50-channel digital voltmeter is illustrated in Figure 10, which provides either printout or a graph on an X-Y plotter. The 50-pin plug-in box is illustrated in the insert.

An X-Y polarization curve plotter is illustrated in the center of Figure 10. The polarization curve plotter is described in detail in Section VIII of this report.

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XIII CORPORATE FUNDED RESEARCH PROGRAM

During the past quarter, investigations have been conducted under the Corporate Funded Research Program in the following general areas:

1. Construction, installation, and calibration of electronic and mechanical equipment
2. Obtaining manufacturers' literature concerning pumps, flow meters, separators, structural materials, and electrode materials
3. Reproducibility of data
4. Use of subculturing (transfer) methods to increase electrical power output
5. Effect of electrode area on current density.

Equipment construction and installation have included a 50-channel digital voltmeter for periodically recording voltage and current; X-Y plotters for polarization curves; glass and plastic cells; manifolds for bubbling oxygen and helium over cathodes and anodes, respectively; and Luggin capillaries (salt bridges) with calomel electrodes.

The reproducibility of electrical output from biofuel cells has been found to vary widely. It is anticipated that much of the experimental work being conducted will improve the reproducibility.

Subculturing techniques have been tried in only one experiment, to maintain electrical output at an optimum level. In the first test, the electrical output was neither increased nor decreased.

The Corporate Funded Research Program provided considerable aid in the search for bio-anode organisms for use with human fecal material, as well as bio-cathode organisms that will reduce nitrate ion.

A test was made in an H-cell to determine the effect of electrode area on current density. The electrolyte was saturated KCl. At very low current densities (below 6×10^{-4} amperes per square foot, based on one side), there was no difference in the current densities, whether the electrode had only one side or both sides exposed. Above that current density, two sides conducted better than one side, up to a current density of 8×10^{-3} amperes per square foot. At still higher current densities, an electrode with only one side exposed conducted better than one with both sides exposed, possibly because of conflicting electrolytic current paths.

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XIV FUTURE WORK

Investigations will be conducted on the following topics:

A. Improvement of reproducibility of electrical power output

B. Fabrication of a multiple cell experimental biofuel cell system, after a reasonably high degree of reproducibility of power output has been attained

C. Evaluation of hydrogen or ammonia as fuels, to determine the kinetics and electrical power output of such systems under varying conditions

D. Selection or revision of choice of bio-anode micro-organisms

E. Determination of optimum composition of human waste for use as a biofuel

F. Pretreatment and preculturing of human waste to optimize electrical power output

G. Obtaining polarization and power curves from the most promising systems

H. Determination of effects of structural materials on biochemical activity

I. Construction and installation of constant voltage and constant current sources, to be used in determining electrochemical parameters of the biochemical fuel cells.

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XVI APPENDIXES

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APPENDIX A

SUMMARY OF BIO-ANODE EXPERIMENTS

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Figure 1 illustrates the results of some of the early screening experiments. The graph shows the best open-circuit potentials and short-circuit currents obtained from each system. Details concerning the experiments are presented in the following paragraphs. In each case, the type of separator and cell used, the media employed as fuel-anolyte mixture and catholyte, and the micro-organisms added (if any) are described. Formulae for the appropriate media are listed in Tables II through VI; all media were sterile. Feces and urine were of human origin and non-sterile unless otherwise indicated.

All readings of voltage and current were of short duration (less than two seconds) and were intermittent; they were not steady-state values under sustained loads.

Glass H-Cells with Sintered Glass-Agar Plugs; Platinized Platinum Foil Electrodes

Experiment 1: Fuel-anolyte contained Desulfovibrio desulfuricans, 3 percent feces, 28 percent triple filtered sea water (final filtration was with 0.45μ Millipore filter paper), and 69 percent lactate medium. Anode mixture was pre-incubated at 37°C for 3 days. Catholyte was lactate medium.

Experiment 2: Essentially the same as Experiment 1, except that the catholyte was liquid Starkey medium. Both anode and cathode mixtures were pre-incubated at 37°C for 3 days.

Experiment 3: Essentially the same as Experiment 1, except that the added electrolyte was sulfate medium. The catholyte was nitrate medium; a micro-organism was used (Escherichia coli). Both anode and cathode mixtures were pre-incubated at 37°C for 3 days.

Experiment 4: Essentially the same as Experiment 3, except that the cathode micro-organism was Bacillus subtilis.

Experiment 5: Fuel-anolyte contained 10 percent (by weight) feces, 10 percent urine, and 80 percent triple filtered sea water (final filtration was with 0.45μ Millipore filter paper).

Experiment 6: Fuel-anolyte contained 5 percent feces, 5 percent urine, and 90 percent sulfate medium. pH was maintained at 6.0 to 7.0. Catholyte was sulfate medium.

Experiment 7: Essentially the same as Experiment 6, except that air was bubbled continuously through the mixture. The mixture was maintained at 42.5°C, except that electrical properties were obtained at room temperature.

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Experiment 8: Fuel-anolyte contained 5 percent (by weight) feces, 5 percent urine, and 90 percent sterile lactate medium, and was incubated at 37°C for 24 hours. Catholyte was lactate medium.

Experiment 9: Same as Experiment 8, except that sterile liquid Starkey medium was used instead of lactate medium.

Experiment 10: Same as Experiment 8, except that sterile distilled water was used instead of lactate medium.

Experiment 11: Same as Experiment 8, except that triple filtered sea water (final filtration with 0.45μ Millipore filtered paper) was used instead of lactate medium.

Experiment 12: Fuel-anolyte mixture contained feces and urine in the weight ratio 3:4, to provide a 1 percent (by weight) fuel mixture, and Escherichia coli was added. The medium was D-17.

Experiment 13: Same as Experiment 12, except that a cathode micro-organism was used (Escherichia coli).

Experiment 14: Same as Experiment 12, except that no Escherichia coli was added to the fuel-anolyte.

Experiment 15: Same as Experiment 14, except that a cathode micro-organism was used (Escherichia coli).

Experiment 16: Fuel-anolyte mixture contained Desulfovibrio desulfuricans (DSV), 1 percent feces, 1 percent urine, and 98 percent sulfate media. A cathode micro-organism (Escherichia coli) was used in sterile nitrate medium.

Experiment 17: Same as Experiment 16, except without feces in the fuel-anolyte mixture.

Experiment 18: Same as Experiment 16, except without urine in the fuel-anolyte mixture.

Experiment 19: Same as Experiment 16, except without cathode micro-organism.

Experiment 20: Fuel-anolyte mixture contained Escherichia coli, 1 percent feces, and 99 percent sterile sulfate medium. The cathode mixture was sterile nitrate medium without micro-organisms.

Experiment 21: Fuel-anolyte mixture contained 1 percent feces, 1 percent urine, and the fungus Linderina in sterile sulfate medium. The

cathode was nitrate medium.

Experiment 22: Same as Experiment 21, except without urine.

Experiment 23: Same as Experiment 21, except without feces.

Experiment 24: Fuel-anolyte mixture contained 1 percent feces (by weight) and Escherichia coli in sulfate medium; catholyte was nitrate medium.

Experiment 25: Same as Experiment 24, except that the fuel-anolyte mixture was pre-incubated at 37°C for 24 hours.

Experiment 26: Same as Experiment 24, except that the feces was sterilized before being added to the sulfate medium.

Experiment 27: Same as Experiment 24, except that the feces-sulfate mixture was sterilized after homogenizing.

Experiments 28 and 29: Same as Experiment 26, except that greater care was used in maintaining sterility.

Experiment 30: Fuel-anolyte contained 10 percent (by weight) feces in urine; catholyte was nitrate medium.

Experiment 31: Same as Experiment 30, except 0.1 percent (by weight) each of Na_2SO_4 and NH_4Cl were added to the fuel-anolyte.

Experiment 32: Same as Experiment 30, except that sulfate medium was used instead of urine.

Experiment 33: Same as Experiment 30, except that the fuel-anolyte contained 65 percent (by weight) sulfate medium and 25 percent triple filtered sea water (final filtration with 0.45μ Millipore filter paper) instead of urine.

Experiment 34: Same as Experiment 30, except that the fuel-anolyte contained 90 percent (by weight) sulfate medium and Escherichia coli instead of urine.

Experiment 35: The fuel-anolyte was urine, the catholyte nitrate medium.

Experiment 36: Fuel-anolyte was urine, to which 0.1 percent (by weight) each of Na_2SO_4 and NH_4Cl were added. The catholyte was nitrate medium.

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Experiment 37: Fuel-anolyte was 10 percent (by volume) urine in 90 percent sulfate medium. The catholyte was nitrate medium.

Experiment 38: Fuel anolyte contained 10 percent (by weight) urine, 25 percent triple filtered sea water (final filtration through 0.45μ Millipore paper), and 65 percent sulfate medium. The catholyte was nitrate medium.

Experiment 39: Fuel-anolyte contained 10 percent (by weight) urine, 90 percent sulfate medium, and Escherichia coli; catholyte was nitrate medium.

Experiment 40: Same as Experiment 36, except that 1 milliliter of Pseudomonas denitrificans was added to 100 milliliters of urine.

Experiment 41: Fuel-anolyte contained 10 percent (by weight) feces in urine; the agar plug was made in saturated KCl solution; and the catholyte was 5 percent KCl and 5 percent NaCl in deionized water.

Experiment 42: Same as Experiment 41, except that the agar plug was made in the catholyte solution.

Experiment 43: Same as Experiment 41, except that the fuel-anolyte contained 20 percent feces.

Experiment 44: Same as Experiment 43, except that the agar plug was made in the catholyte solution.

Experiment 45: Same as Experiment 41, except that the fuel-anolyte contained 30 percent feces.

Experiment 46: Same as Experiment 45, except that the agar plug was made in the catholyte solution.

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Plastic, Continuous Flow System; Platinized Platinum Screen Electrodes

Experiment 47: Fuel-anolyte contained 25 percent (by weight) feces, 25 percent urine, and 50 percent sulfate medium. Catholyte was non-biological (air) in sulfate medium. Separator was cellulose acetate.(1)

Experiment 48: Same as Experiment 47, except for the separator.

a) Separator was cellulose acetate.(1)
b) Separator was anion exchange membrane.(2)

Experiment 49: Same as Experiment 48, to check reproducibility.

Experiment 50: Same as Experiment 47, except for the separator.

a) Separator was cellulose acetate.(1)
b) Separator was cation exchange membrane.(3)

(1) Cellulose acetate was E. H. Sargent Co. S-14825, 0.001 inch thick.

(2) Anion exchange membrane was Ionics, Inc., Nepton AR111A, 0.024 inch thick.

(3) Cation exchange membrane was Ionics, Inc., Nepton Cr-61, 0.024 inch thick.

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FIGURE 1

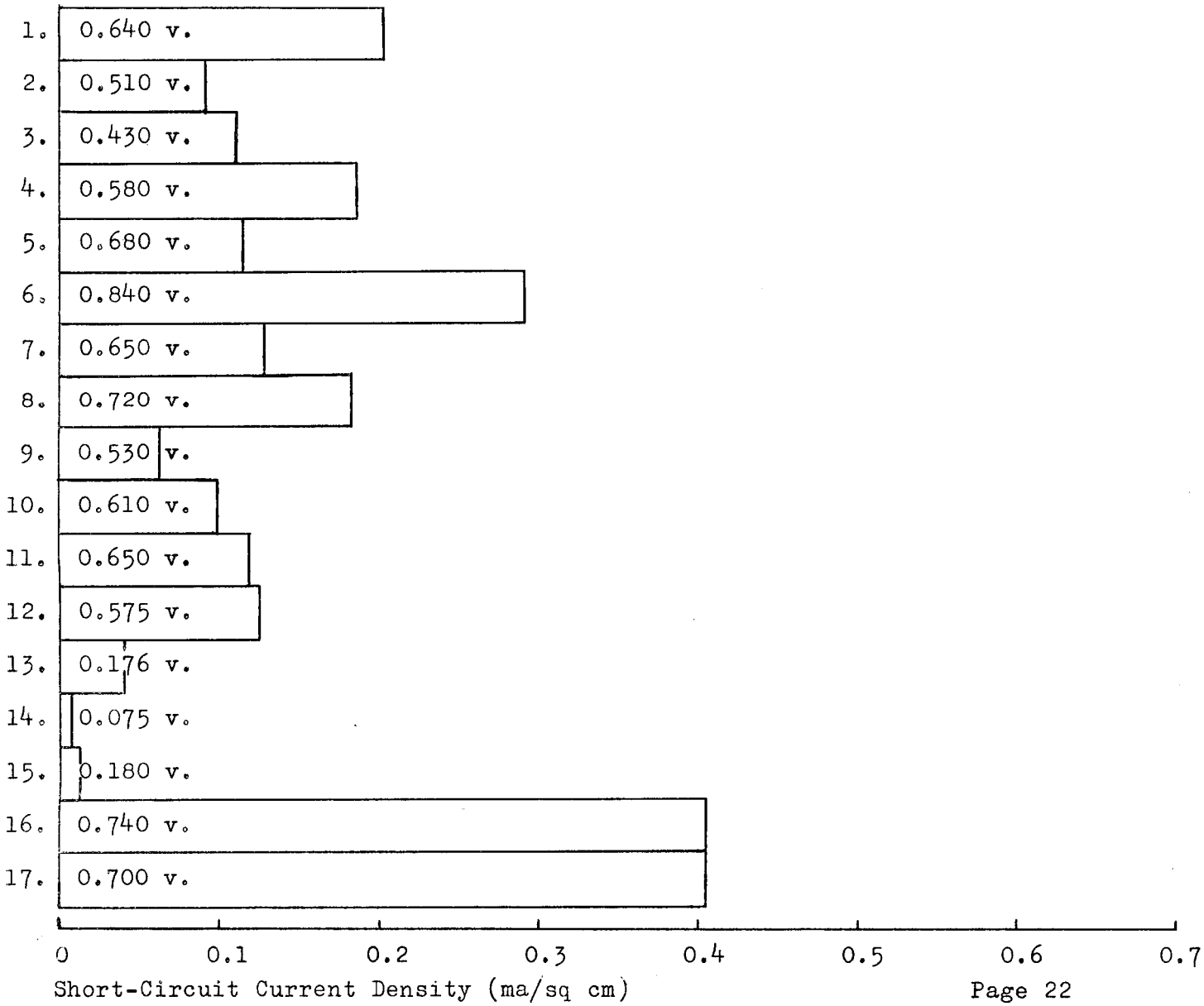
Summary of Experiments

Determination of Fuel-Oxidant Compositions

ELECTRODES:

	GEOMETRIC AREA (Sq. cm)
a) H-CELL: Platinized Pt foil, 1 square inch on each side, both sides exposed	6.45
b) PLASTIC: Platinized Pt screen, 80 mesh, 2-1/8 inches diameter exposed	22.8

Open-circuit potentials were measured anode-cathode



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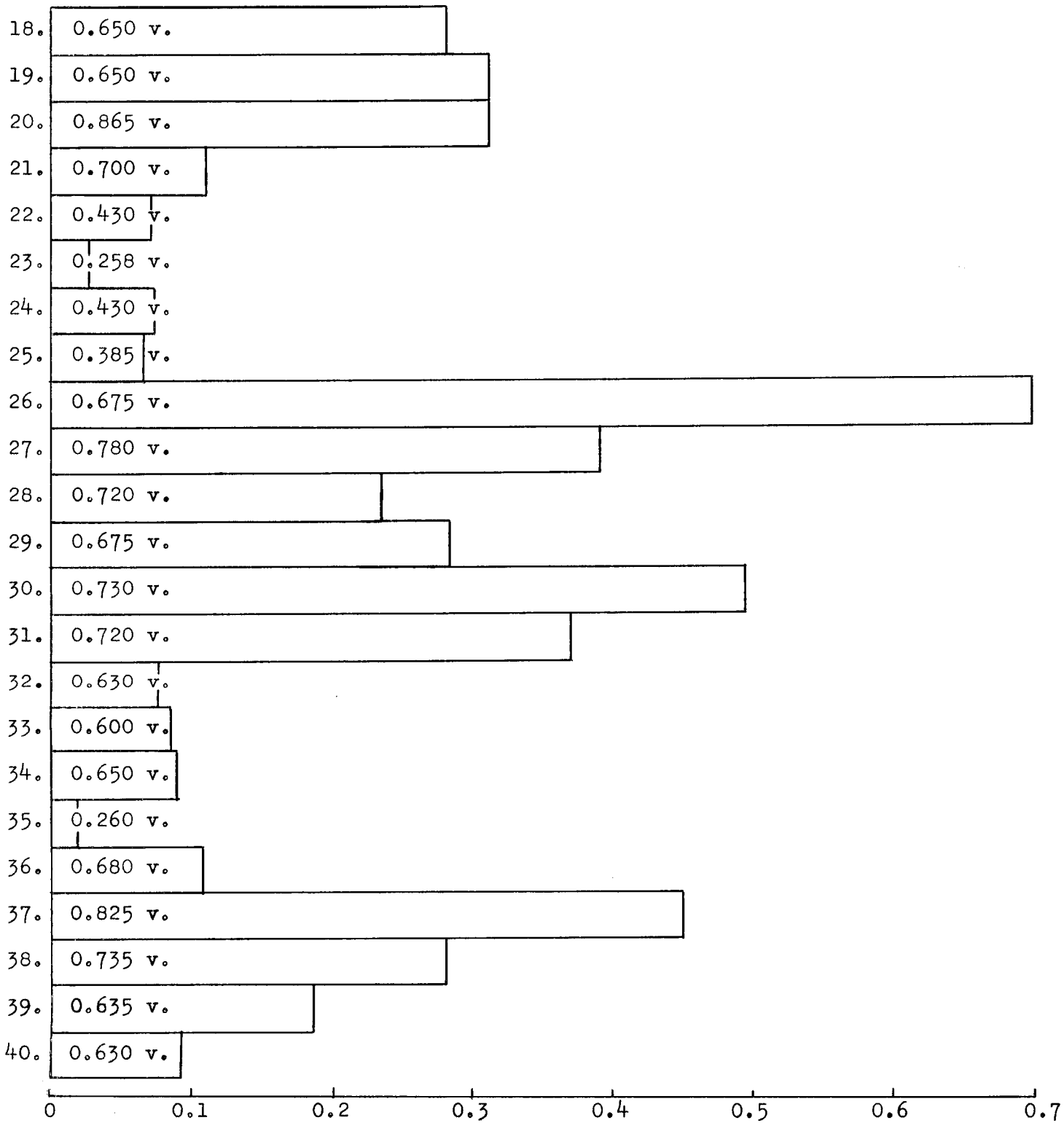
Determination of Fuel-Oxidant Compositions (continued)



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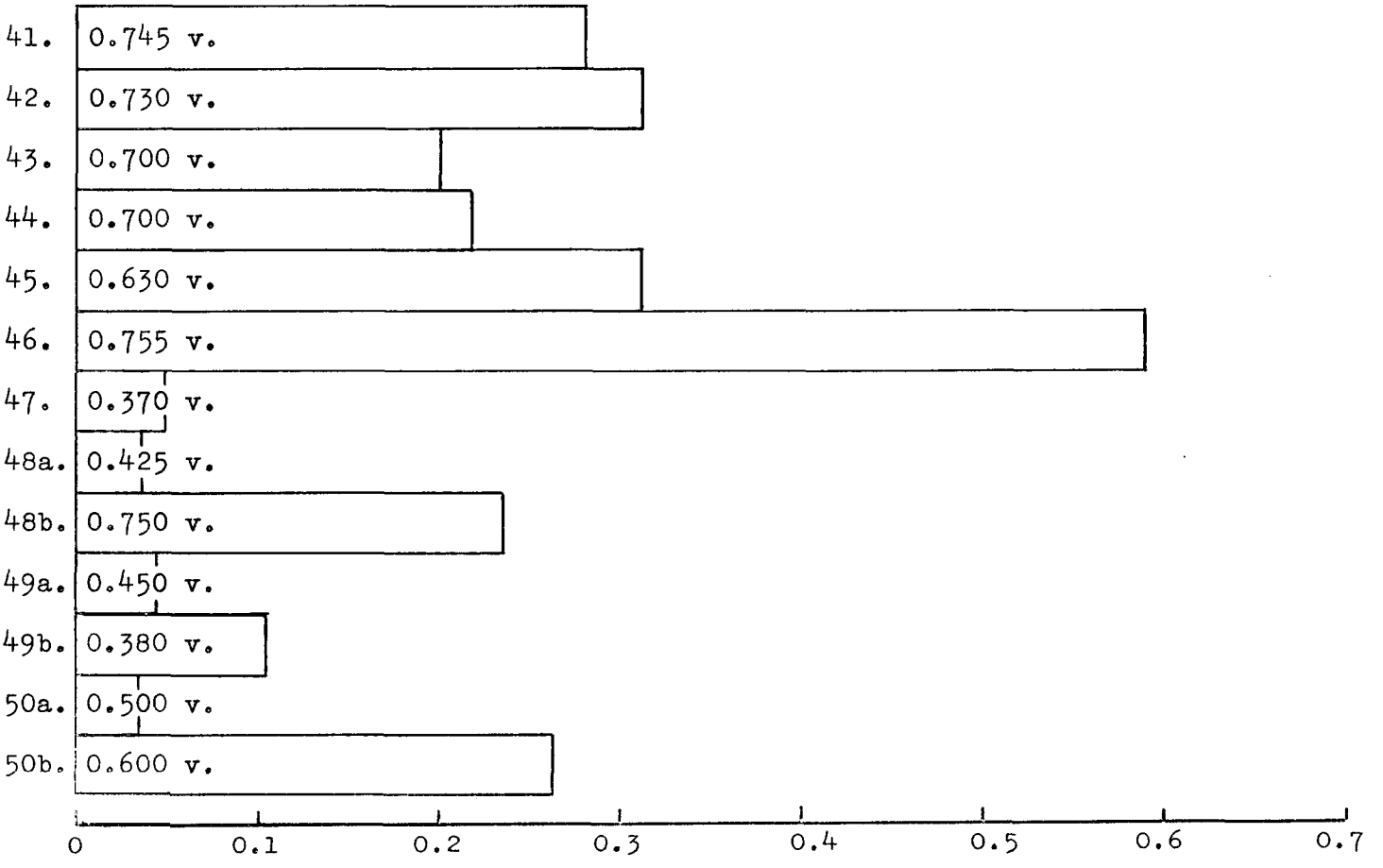
Ref: 295/885-21/2928

Determination of Fuel-Oxidant Compositions (continued)



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APPENDIX B

PRETREATMENT OF WASTE

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The experiments regarding the pretreatment of human waste are described in detail in the following paragraphs. All readings were taken at room temperature, thus avoiding temperature effects on electrical properties. Results of these experiments are presented in Table I.

P-1. Fuel-anolyte: 10 percent (by weight) feces, 10 percent urine (both non-sterile), and 80 percent triple filtered sea water (final filtration with 0.45μ Millipore paper); homogenized. Catholyte: non-biological (air), triple filtered sea water. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 37°C. Storage vessel: stainless steel (type 304).

P-2. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table III), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 37°C. Storage vessel: stainless steel (type 304). pH adjusted to 6.5.

P-3. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table III), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 42.5°C. Storage vessel: stainless steel (type 304). pH adjusted to 6.0, and air was bubbled continuously into the storage vessel.

P-4. Fuel anolyte: 5 percent (by weight) feces (non-sterile) and 95 percent sterile sulfate medium (see Table III), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 37°C. Storage vessel: stainless steel (type 304). Air was bubbled continuously into the storage vessel.

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P-5. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table III), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug.

a. Temperature: 23°C. Storage vessel: glass H-cell.
b. Temperature: 40°C. Storage vessel: glass H-cell.
c. Temperature: 40°C. Storage vessel: stainless-steel (type 304).

P-6. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), in 90 percent sterile sulfate medium (see Table III), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature 23°C. (room temperature).

a. Storage vessel: glass H-cell.
b. Storage vessel: stainless steel (type 304).

P-7. Fuel-anolyte: 30 grams of feces in 100 grams of urine (both non-sterile). Catholyte: non-biological (air), in aqueous salt solution (5 percent NaCl - 5 percent KCl by weight). Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: cellulose acetate. Temperature: room temperature: 23°C. Storage vessel: glass bottle.

These experiments indicate that there is a general increase in open-circuit potential and in short-circuit current for 3 to 24 hours, followed by a slow decrease in electrical power output, sometimes attaining a psuedo-plateau after a total time of about 48 hours. Maintaining either the pH below 7 or aerobic conditions (bubbling with air), seemed to be detrimental (i.e., less open-circuit potential). The determination of the effect of temperature requires further experimentation. There has been some indication that pretreatment in the stainless steel (type 304) vessel may be less effective than in glass; however, a single experiment conducted thus far, entirely in glass, to resolve this problem has produced unsatisfactory results. These experiments will be continued, and if necessary, the storage vessel will be lined with chemically and bacterially resistant material.

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TABLE I

Pretreatment of Waste

Open-circuit potentials measured were anode versus cathode.

P-1

Time (hours)	0	24	48	120
Open-circuit potential (v.)	0.145	0.638	0.475	0.425
Short-circuit current density (ma/sq.cm)	0.070	0.070	0.062	0.047

P-2

Time (hours)	0	24	48	120	144	168
Open-circuit potential (v.)	0.148	0.660	0.480	0.580	0.475	0.600
Short-circuit current density (ma/sq.cm)	0.008	0.093	0.078	0.085	0.037	0.101

P-3

Time (hours)	0	72	96	120
Open-circuit potential (v.)	0.175	0.470	0.140	0.120
Short-circuit current density (ma/sq.cm)	0.031	0.078	0.039	0.023

P-4

Time (hours)	0	24	68	96	120
Open-circuit potential (v.)	0.290	0.240	0.260	0.290	0.270
Short-circuit current density (ma/sq.cm)	0.062	0.050	0.054	0.047	0.031

P-5a

Time (hours)	0	24	48	144	168	192	216
Open circuit potential (v.)	0.110	0.210	0.730	0.590	0.525	0.350	0.290
Short-circuit current density (ma/sq.cm)	0.108	0.326	0.715	0.620	0.620	0.216	0.216

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TABLE I (continued)

P-5b

Time (hours)	0	24	48	144	168	192	216
Open-circuit potential (v.)	0.110	0.360	0.475	0.210	0.110	0.130	0.085
Short-circuit current density (ma/sq cm)	0.108	0.620	0.404	0.144	0.093	0.078	0.062

P-5c

Time (hours)	0	24	48	144	168	192	216
Open-circuit potential (v.)	0.110	0.550	0.325	0.300	0.450	0.445	0.440
Short-circuit current density (ma/sq cm)	0.108	0.744	0.216	0.205	0.295	0.186	0.216

P-6a

Time (hours)	0	24	48	72	96	168	192	216
Open-circuit potential (v.)	0.140	0.760	0.835	0.680	0.680	0.525	0.250	0.125
Short-circuit current density (ma/sq cm)	0.066	0.139	0.357	0.357	0.171	0.093	0.047	0.012

P-6b

Time (hours)	0	24	48	72	96	168	192	216
Open-circuit potential (v.)	0.160	0.285	0.450	0.540	0.420	0.580	0.440	0.575
Short-circuit current density (ma/sq cm)	0.066	0.033	0.116	0.155	0.078	0.139	0.316	---

P-7

Time (hours)	0	27	51	148	168
Open-circuit potential (v.)	0.520	0.550	0.425	0.450	0.400
Short-circuit current density (ma/sq cm)	0.002	0.002	0.002	0.001	0.001

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APPENDIX C

DATA AND APPARATUS

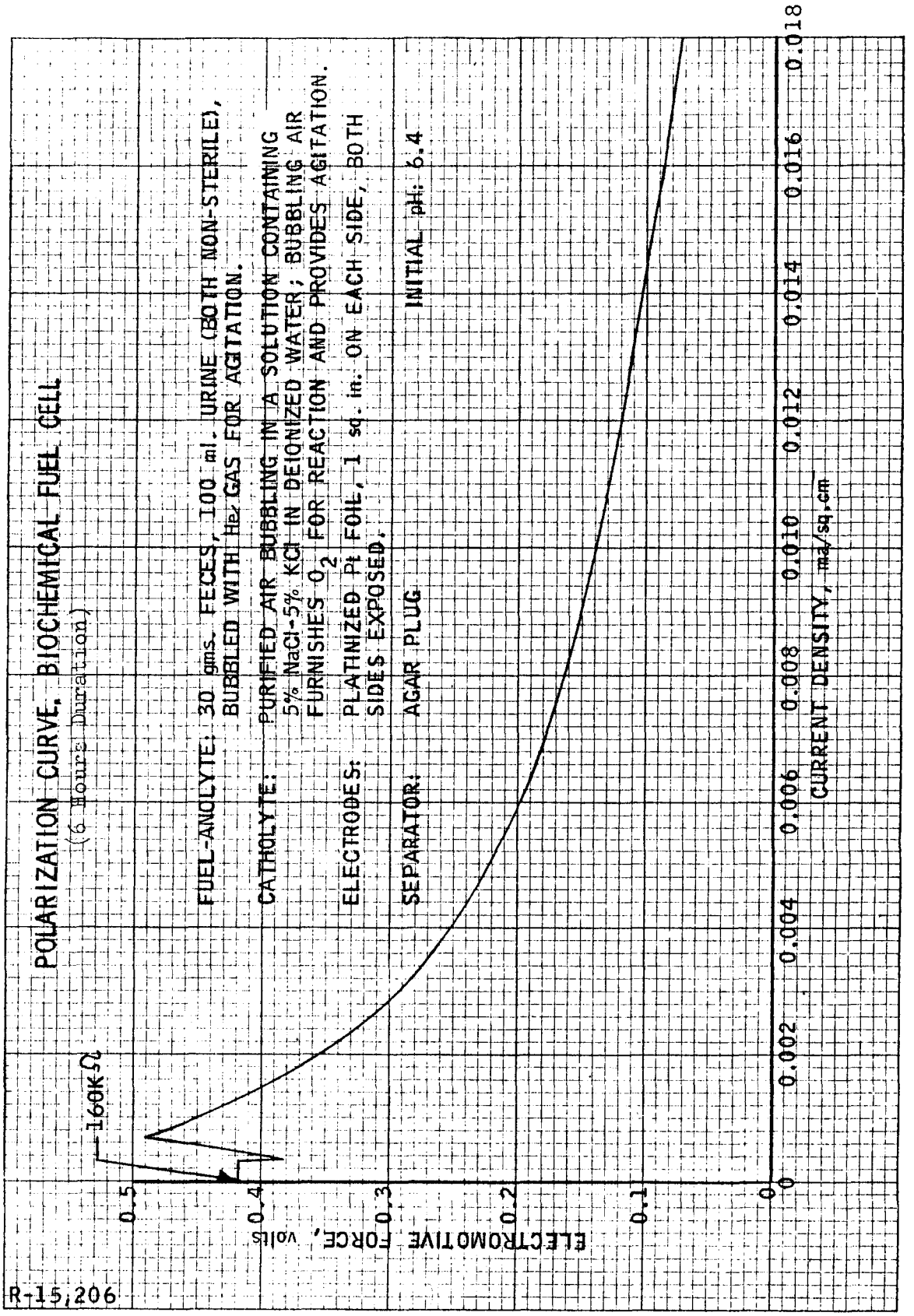

POLARIZATION CURVE, BIOCHEMICAL FUEL CELL
(6 Hours Duration)
160KΩ
FUEL-ANOLYTE: 30 gms. FECES, 100 ml. URINE (BOTH NON-STERILE), BUBBLED WITH He GAS FOR AGITATION.
CATHOLYTE: PURIFIED AIR BUBBLING IN A SOLUTION CONTAINING 5% NaCl-5% KCl IN DEIONIZED WATER; BUBBLING AIR FURNISHES O_2 FOR REACTION AND PROVIDES AGITATION.
ELECTRODES: PLATINIZED Pt FOIL, 1 sq. in. ON EACH SIDE, BOTH SIDES EXPOSED.
SEPARATOR: AGAR PLUG
INITIAL pH: 6.4
ELECTROMOTIVE FORCE, volts
0.5
0.4
0.3
0.2
0.1
0
0 0.002 0.004 0.006 0.008 0.010 0.012 0.014 0.016 0.018
CURRENT DENSITY, ma/sq.cm
R-15,206

Figure 2

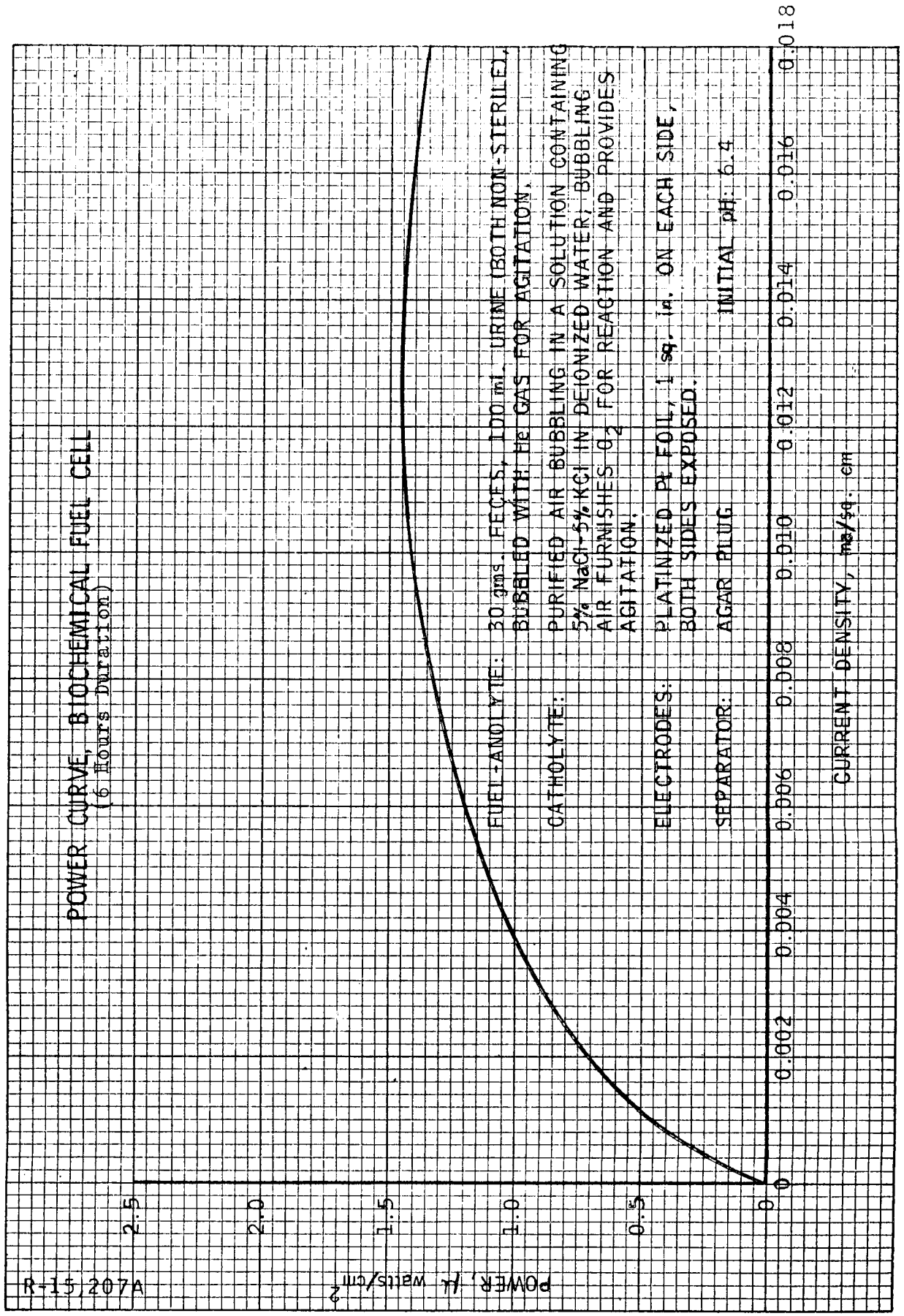
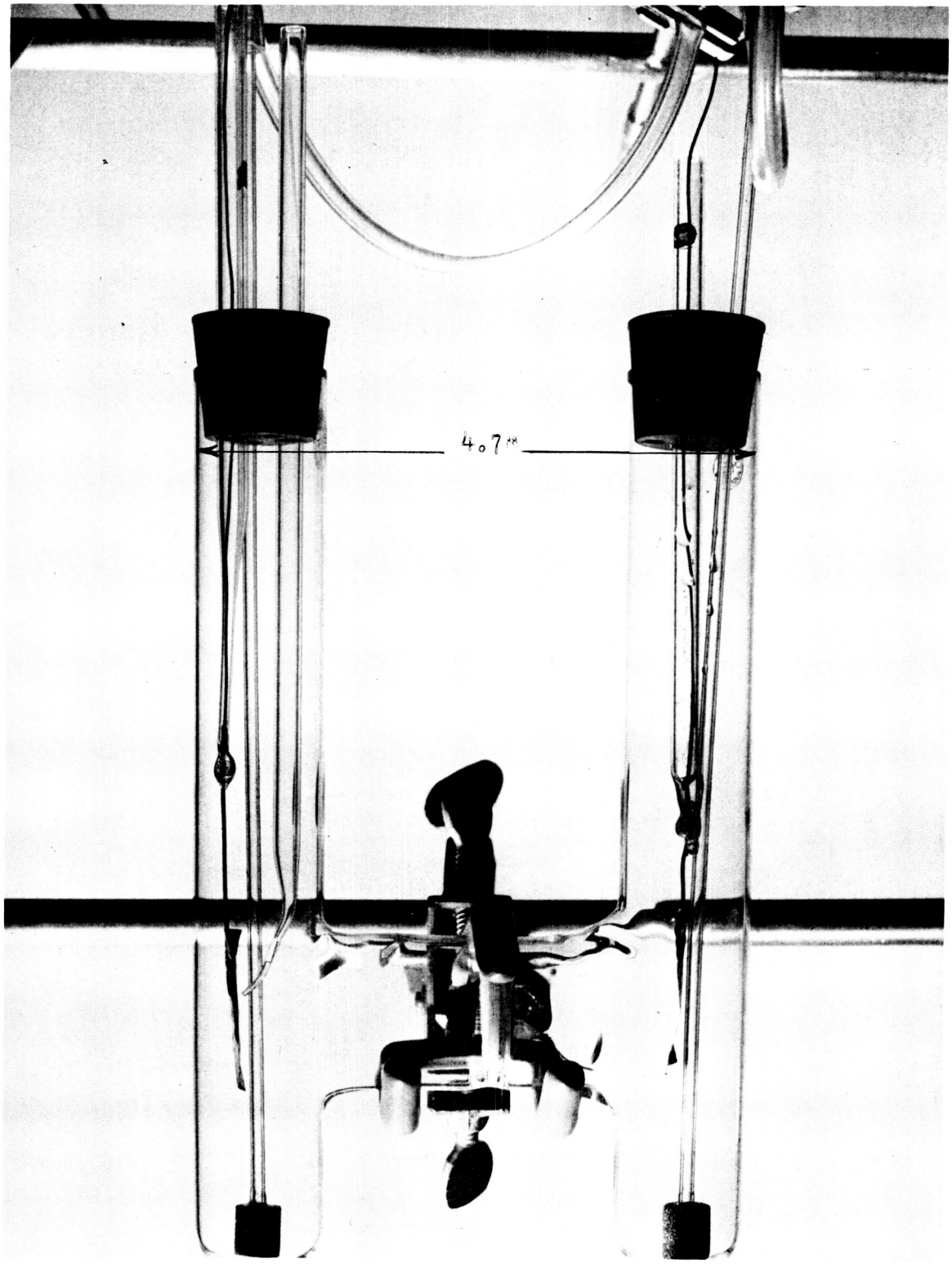

POWER CURVE, BIOCHEMICAL FUEL CELL
(6 Hours Duration)
FUEL-ANOLYTE: 30 gms. FECES, 100 ml. URINE (BOTH NON-STERILE), BUBBLED WITH N_2 GAS FOR AGITATION.
CATHOLYTE: PURIFIED AIR BUBBLING IN A SOLUTION CONTAINING 5% NaCl-5% KCl IN DEIONIZED WATER; BUBBLING AIR FURNISHES O_2 FOR REACTION AND PROVIDES AGITATION.
ELECTRODES: PLATINIZED Pt FOIL, 1 sq. in. ON EACH SIDE, BOTH SIDES EXPOSED.
SEPARATOR: AGAR PLUG
INITIAL pH: 6.4
POWER, P, watts/cm²
CURRENT DENSITY, ma/sq. cm
0
0.5
1.0
1.5
2.0
2.5
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
0.018
R-1$,207A

Figure 3

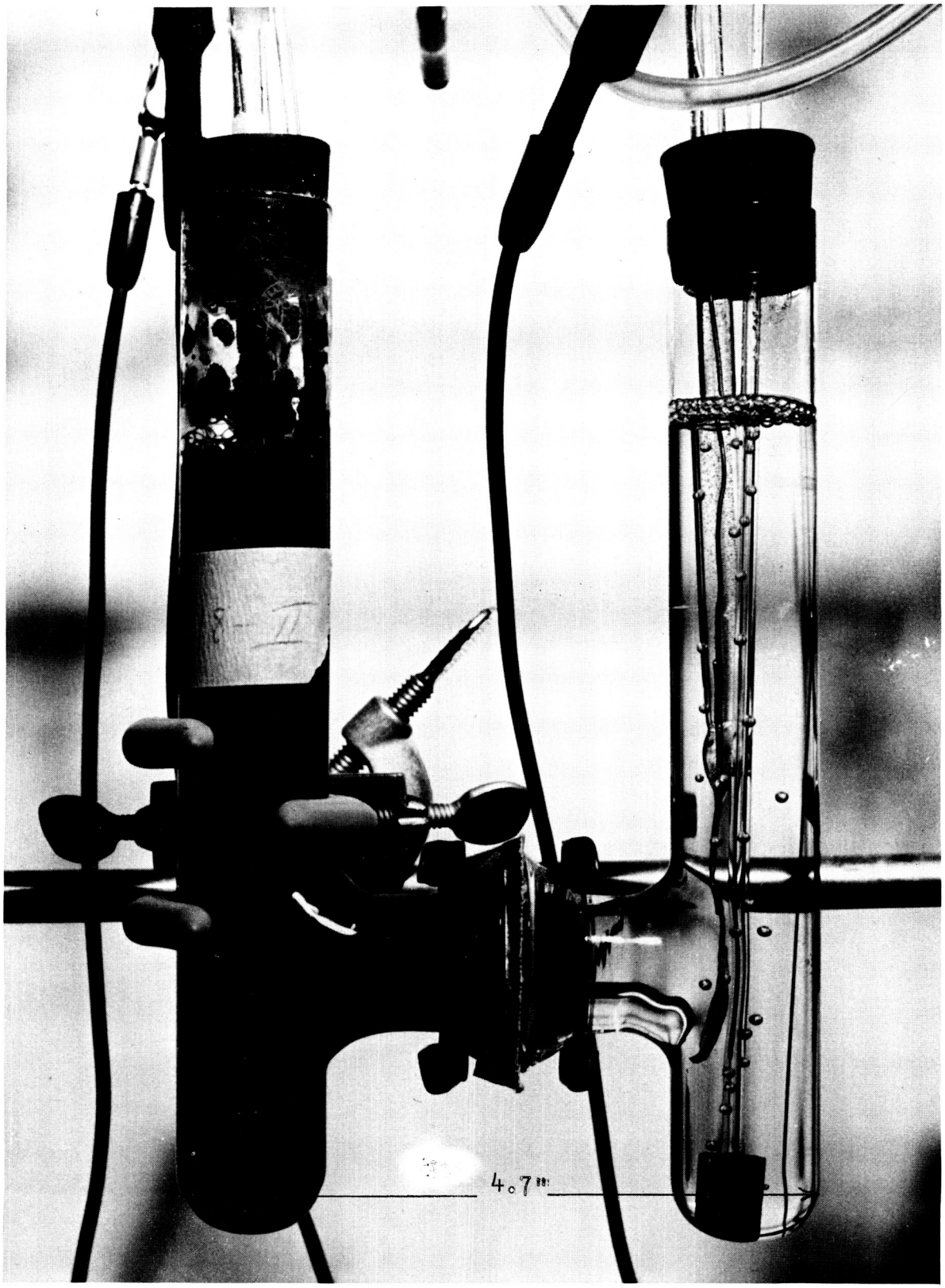
H-CELL; AQAR PLUG TYPE (CLOSEUP)



NEG. 4831-4

Figure 4

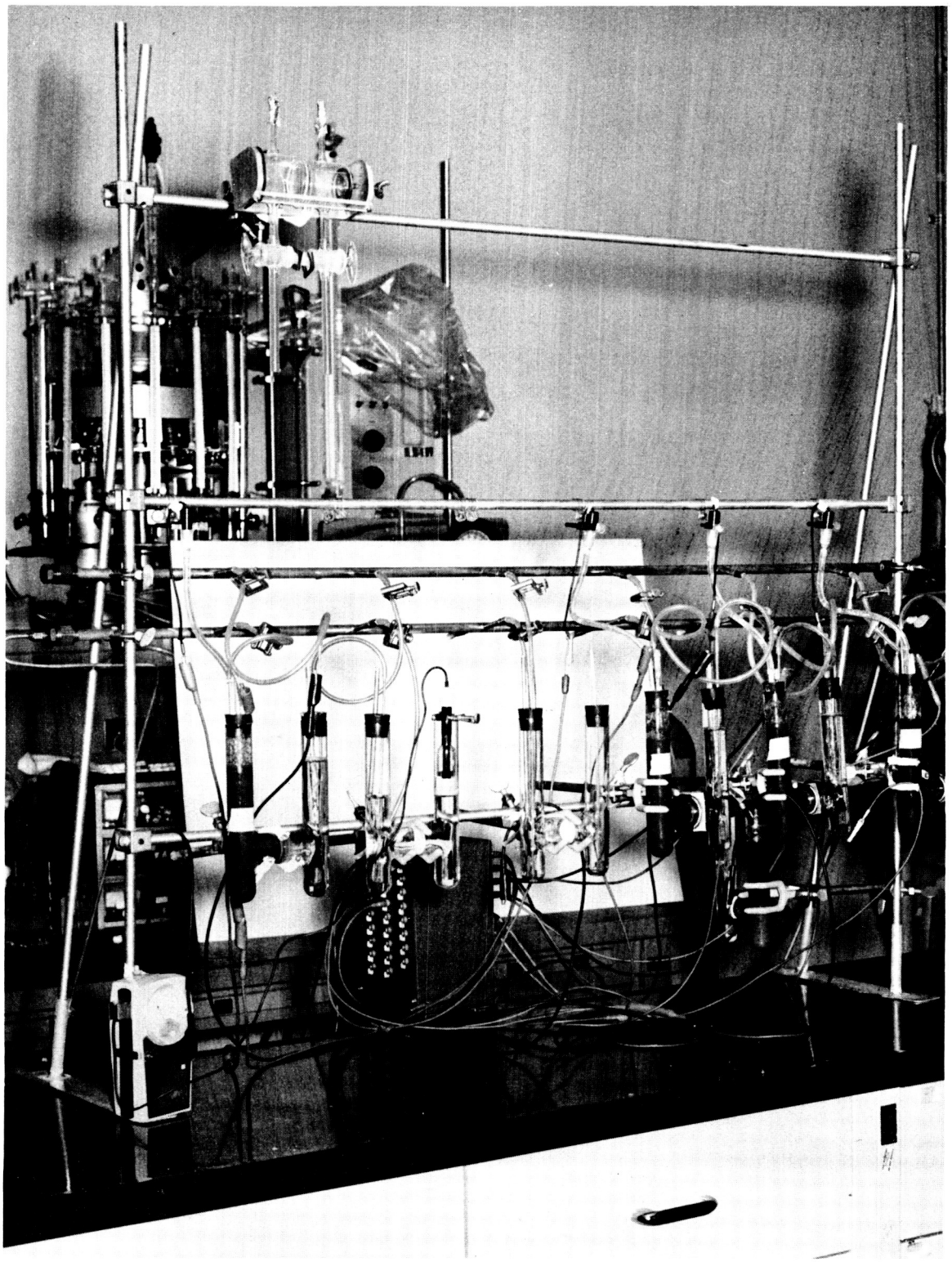
H-CELL; O-RING JOINT TYPE (CLOSEUP)



NEG. 4831-3

Figure 5

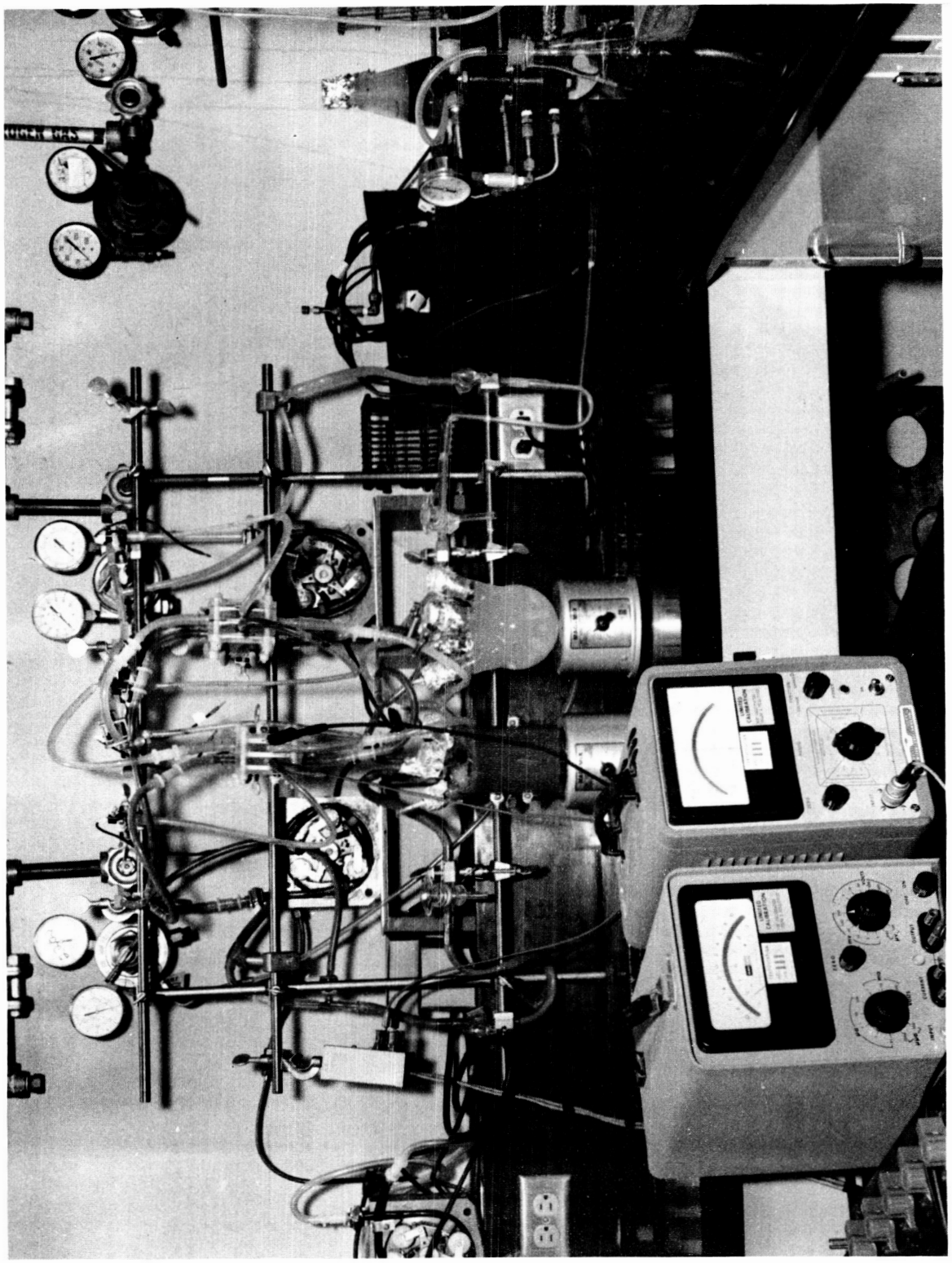
H-CELL TEST ASSEMBLY



NEG. 4831-1

Figure 6

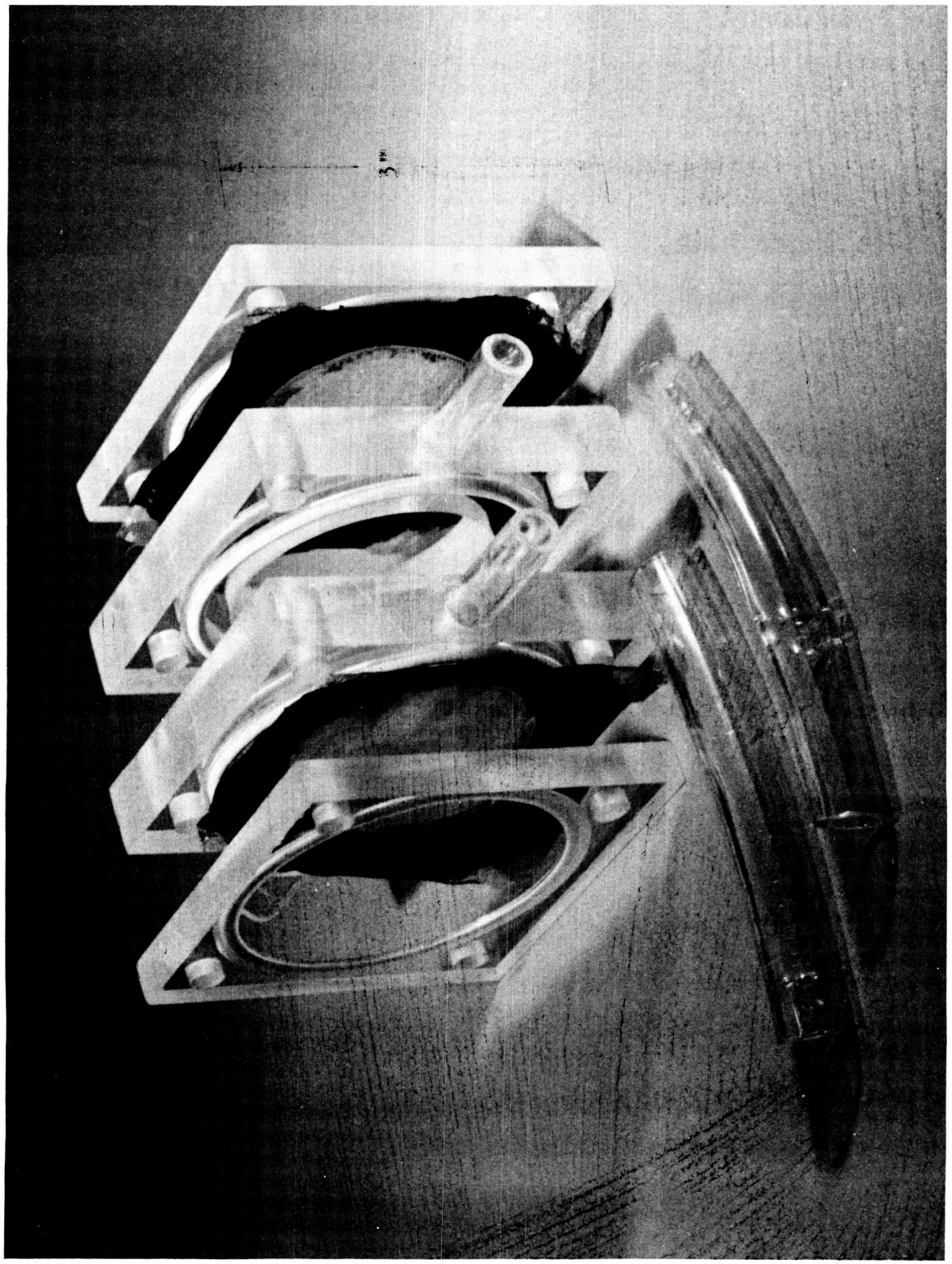
BIOFUEL CELL, CONTINUOUS FLOW SYSTEM



NEG. 4805-1

Figure 7

PLASTIC CELL, DISMANTLED



NEG. 4702-31CN

Figure 8

A-C CONDUCTIVITY MEASUREMENT EQUIPMENT

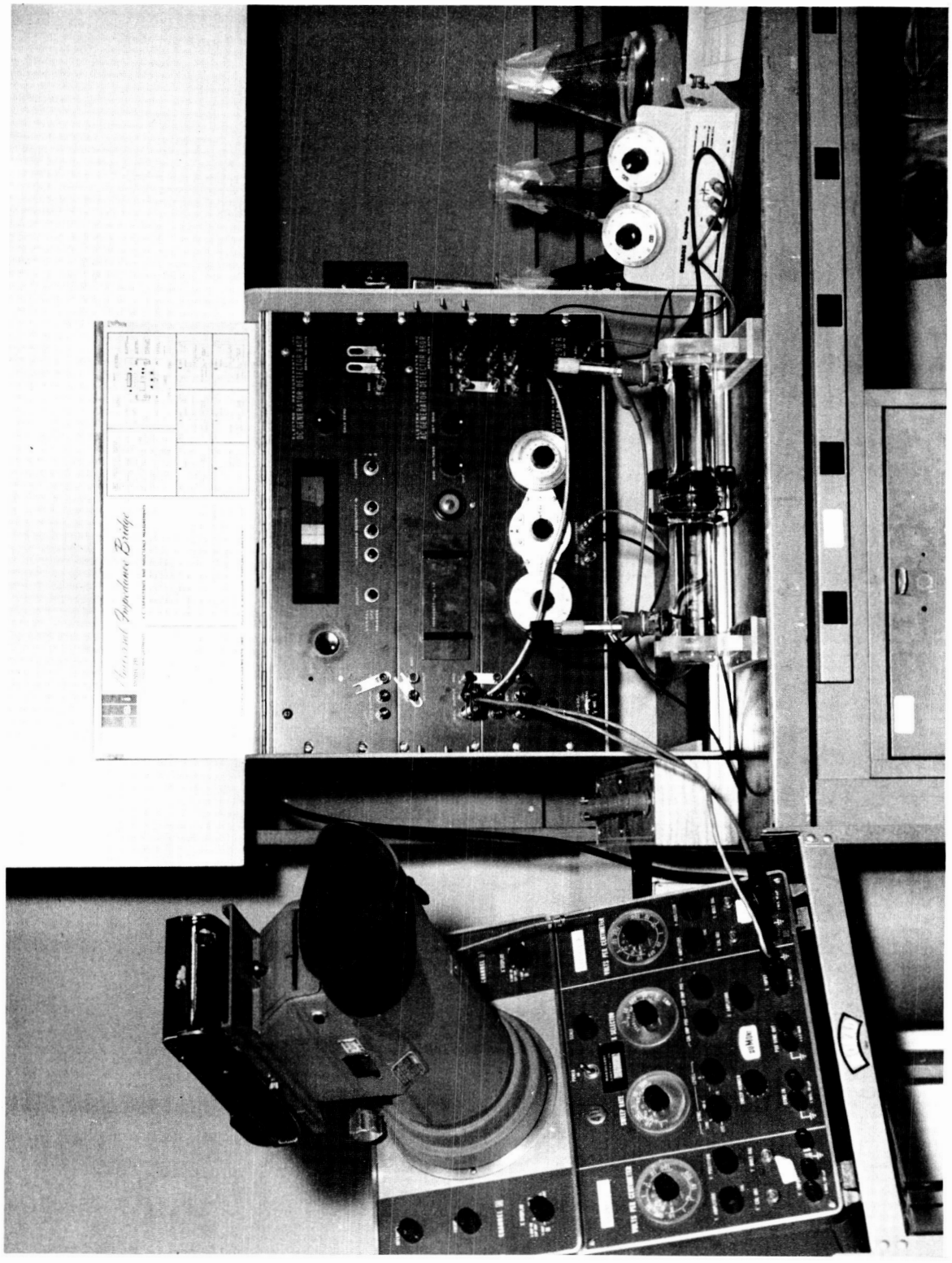


Figure 9

FUEL CELL DATA READOUT SYSTEM

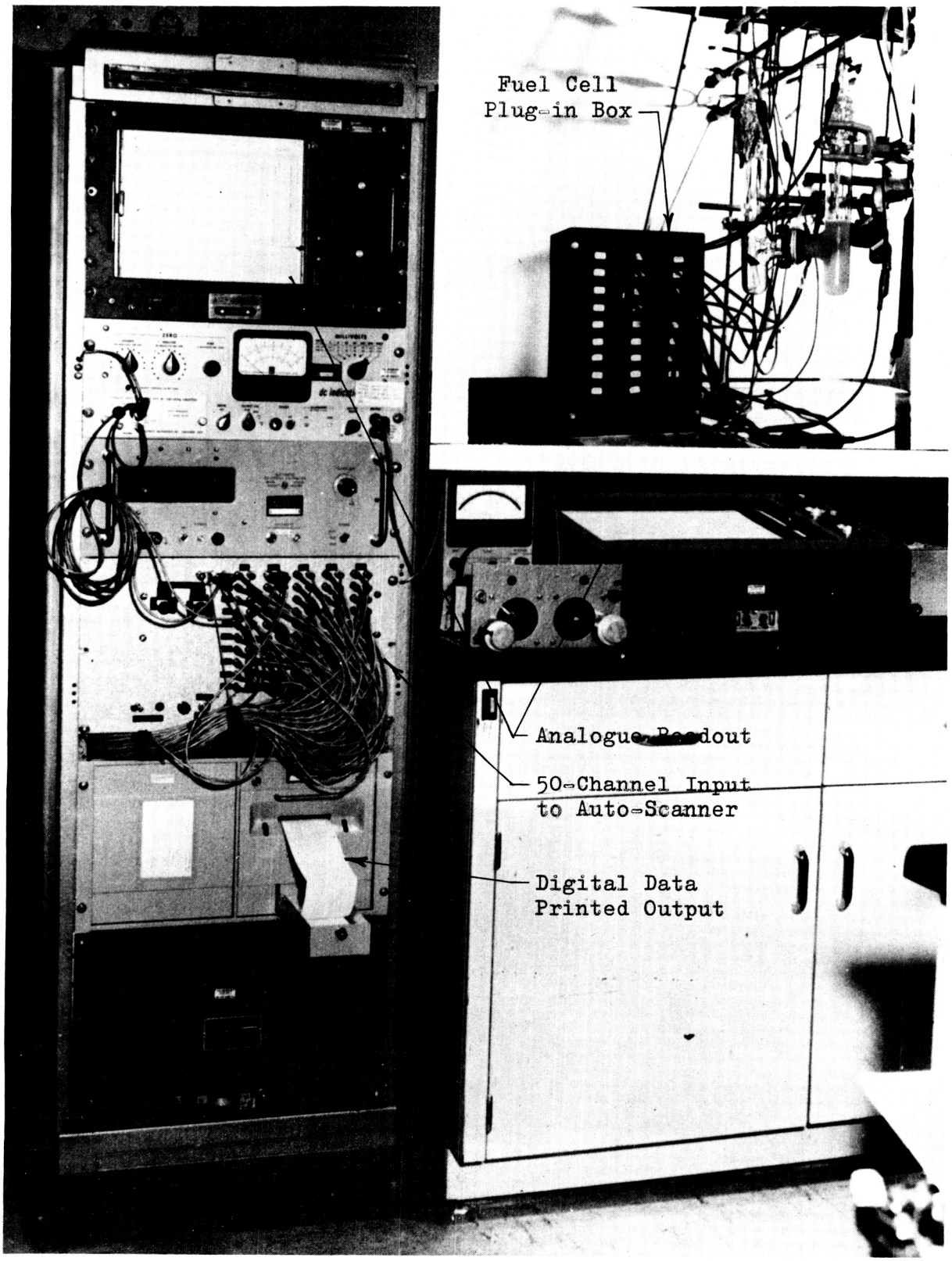


Figure 10

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TABLE II

Composition of Lactate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2 gm
K_2HPO_4	1.0
$FeSO_4 \cdot 7H_2O$	50.0
$CaCl_2$	20.0
$MnCl_2 \cdot 2H_2O$	2.0
$NaMoO_4 \cdot 2H_2O$	1.0
NH_4Cl	1.0
KNO_3	1.0
$CaCO_3$	2.0
Na lactate	2.0

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TABLE III

Composition of Liquid Starkey Medium

(The following ingredients are added to 500 milliliter tap water)

Peptone	2.5 gm
Beef extract	1.5
Yeast extract	0.1
$MgSO_4 \cdot 7H_2O$	1.5
Na_2SO_4	0.75
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.1
Glucose (dextrose)	2.5

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TABLE IV

Composition of Sulfate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2	gm
K_2HPO_4	1.66	
KH_2PO_4	1.0	
$FeSO_4 \cdot 7H_2O$	0.005	
$MnCl_2 \cdot 2H_2O$	0.002	
$HmoO_4$	0.001	
KNO_3	1.0	
Na lactate	5.0	
$CaCl_2$	0.002	
Na_2SO_4	1.0	
NH_4Cl	1.0	

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TABLE V

Composition of Nitrate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2 gm
K_2HPO_4	1.66
KH_2PO_4	1.0
$FeSO_4 \cdot 7H_2O$	0.005
$MnCl_2 \cdot 2H_2O$	0.002
$HMoO_4$	0.001
KNO_3	1.0
Na lactate	5.0
$CaCl_2$	0.002

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TABLE VI

Composition of D-17 Medium
(Basis: 3 liters of medium)

The medium consists of the following eight stock solutions:

1) Sea Water - 2.25 liters, Distilled Water - 511 ml, EDTA - 12 ml of a stock solution which is 50 gm/l in the di-sodium salt. Prepared in a one gallon autoclave bottle.

2) $MgSO_4 \cdot 7H_2O$--7.5 gm in 25 ml Dist. H_2O.

3) KNO_3--6.0 gm in 50 ml Dist. H_2O.

4) KH_2PO_4--3.0 gm in 25 ml Dist. H_2O.

5) K_2HPO_4--3.0 gm in 25 ml Dist. H_2O.

6) $CaCl_2$--0.325 gm in 25 ml Dist. H_2O.

7) H_3BO_3--0.342 gm; $Fe_2(SO_4)_3$ 0.015 gm in 25 ml H_2O.

8) 60 ml of a solution containing:

$ZnSO_4 \cdot 7H_2O$--4.410 gm/l

$MnCl_2 \cdot 4H_2O$--0.700 gm/l

H_2MoO_4 (85%)--0.462 gm/l

$CuSO_4 \cdot 5H_2O$--0.800 gm/l

$Co(NO_3)_2$--0.250 gm/l

The stock solutions are autoclaved separately and allowed to cool to room temperature. Then each solution is added aseptically to solution No. 1, slowly and with vigorous agitation.

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TABLE VII

Fuel-Anolyte Composition
(Non-biological cathode)

EXP. NO.	NON-STERILE FECES (%)	NON-STERILE URINE (%)	MEDIUM (%)		ADDITIONS * (%)		MAXIMUM VOLTAGE ANODE-CATHODE (v.)	MAXIMUM CURRENT DENSITY (ma/sq.cm)
51	0.5	0.5	97	D-17	2	E. coli	0.575	0.124
52	0.5	0.5	99	D-17		---	0.075	0.006
53	1.0	1.0	97	Sulfate	1	DSV	0.650	0.319
54	1.0	---	98	Sulfate	1	E. coli	0.865	0.319
55	1.0	---	98	Sulfate	1	Linderina	0.430	0.070
56	---	1.0	98	Sulfate	1	Linderina	0.355	-----
57	1.0	1.0	97	Sulfate	1	Linderina	0.700	-----
58	1.0	---	98	Sulfate	1	E. coli	0.780	0.388
59	10.0	90.0		---		---	0.730	0.497
60	10.0	89.8	(0.1 Na_2SO_4 (0.1 NH_4Cl			---	0.770	0.465
61	10.0	---	90	Sulfate		---	0.675	0.078
62	10.0	---	(65 (25	Sulfate Seawater		---	0.625	0.093
63	10.0	---	89	Sulfate	1	E. coli	0.700	0.102
64	---	100.0		---		---	0.265	0.037
65	---	99.8	(0.1 Na_2SO_4 (0.1 NH_4Cl			---	0.680	0.115
66	---	10.0	90	Sulfate		---	0.825	0.450
67	---	10.0	(65 (25	Sulfate Seawater		---	0.735	0.279
68	---	10.0	89	Sulfate	1	E. coli	0.635	0.186
69	---	99.0		---	1	P. denitrificans	0.630	0.096

*Based upon normal culture concentration in media.

TABLE VII (continued)

EXP. NO.	FECES (%)	URINE (%)	MEDIUM (%)	ADDITIONS (%)	MAXIMUM VOLTAGE ANODE-CATHODE (v.)	MAXIMUM CURRENT DENSITY (ma/sq.cm)
70	10.0	90.0	---	---	0.760	0.248
71	20.0	80.0	---	---	0.850	0.216
72	30.0	70.0	---	---	0.800	0.590
73	----	100.0	---	---	0.330	0.044
74	----	100.0	---	---	0.825	0.186
75	16.0	84.0	---	---	0.790	0.186
76	16.0	84.0	---	---	0.750	0.202
77	29.0	71.0	---	---	0.615	0.124
78	29.0	71.0	---	---	0.710	0.178
79	23.0	77.0	---	---	0.785	0.682
80	23.0	77.0	---	---	0.730	0.528
81	23.0	77.0	---	---	0.825	1.052
82	23.0	77.0	---	---	0.925	1.365
83	23.0	77.0	---	---	0.810	0.155
84	23.0	77.0	---	---	0.790	0.116
85	23.0	77.0	---	---	0.840	0.248
86	23.0	77.0	---	---	0.730	0.961
87	23.0	77.0	---	---	0.620	0.217
88	23.0	77.0	---	---	0.650	0.186
89	23.0	77.0	---	---	0.700	0.178
90	23.0	77.0	---	---	0.655	0.109
91	22.9	76.7	---	0.4 Cellulase	0.785	0.248

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TABLE VII (continued)

EXP. NO.	FECES (%)	URINE (%)	MEDIUM (%)	ADDITIONS (%)	MAXIMUM VOLTAGE ANODE-CATHODE (v.)	MAXIMUM CURRENT DENSITY (ma/sq.cm)
92	23.0	77.0	---	---	0.680	0.132
93	23.0	77.0	---	---	0.630	0.171
94	23.0	77.0	---	---	0.650	0.209
95	23.0	77.0	---	---	0.655	0.202
96	23.0	77.0	---	---	0.475	0.310
97	23.0	77.0	---	---	0.660	---
98	23.0	77.0	---	---	0.690	---
99	23.0	77.0	---	---	0.750	---
100	23.0	77.0	---	---	0.680	---
101	23.0	77.0	---	---	0.730	---
102	23.0	77.0	---	---	0.675	---

In experiments 54 and 58, above, the difference in values of the potential and current density may have been caused by the fact that the fuel-anolyte mixture of experiment 54 was not sterilized, while the feces and sulfate media of experiment 58 were sterilized after homogenization.

Experiments 73 and 74 are duplicates of each other, as are experiments 75 and 76, and 77 and 78. These experiments were designed to determine whether the electrode must be coated with the biofuel solids of the feces, or whether the electrochemical reaction occurs equally well in the supernatant liquid. In the odd numbered experiments, the electrodes were in the supernatant liquid, while in the even numbered experiments, they were in the settled solids of the feces-urine mixture. Placing the electrodes in the latter position seems to be slightly more effective.

Experiments 79 to 81 were designed to determine the effect of temperature. Experiment 79 was conducted at 12°C, experiment 80 at 24°C, and experiment 81 at 37°C. The effect of temperature was not consistent.

Experiments 82 through 90, and 92 through 102 are based upon the same concentrations of human waste. Differences in the tests involve the separators and electrodes, and they are briefly summarized below:

Experiments	Electrodes	Separator
82, 84, 87*, 100*	Platinized Pt foil	Agar plug
83, 86, 90*, 92*, 99*	Platinized Pt foil	Cellulose acetate(1)
85, 89*, 98*	Platinized Pt foil	Anion exchange(2)
88*, 97*	Platinized Pt foil	Cation exchange(3)
93*, 101*	Platinized Pt foil, 1 side coated	Cellulose acetate(1)
94*, 95*	Platinized Pt screen	Cellulose acetate(1)
96*, 102*	Platinized Ni screen	Cellulose acetate(1)

The high potential and current obtained in experiment 82 were not obtained in the repeated experiments (84, 87, and 100).

The above experiments represent duplicates designed to evaluate electrodes and separators by using the same mixture of fuel-anolyte mixture. Cellulose acetate is a simple and effective separator. Platinized nickel screen may be an effective electrode material.

---

*He was bubbled over anode; air was bubbled over cathode.

(1) Cellulose acetate: E. H. Sargent S-14825, 0.001 in. thick

(2) Anion exchange membrane was Ionics, Inc., AR111A, 0.024 in. thick

(3) Cation exchange membrane was Ionics, Inc., CR-61, 0.024 in. thick

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The following experiments were run at the same times:

82 and 83
84 through 86
87 " 90
92 " 96
97 " 102

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TABLE VIII

Transfer Experiment

ORIGINAL MIXTURE: 30 gms. feces in 100 ml. urine (both non-sterile)

ELECTRODES: Platinized Pt foil, 1 square inch on each side, both sides exposed.

SEPARATOR: Agar plug

CATHOLYTE: Non-biological (air) in aqueous salt solution (5 percent by weight NaCl-5 percent KCl)

I (Original Mixture)

Time (hours)	0	6	72	90	144	234
Open-circuit potential (v.)	0.350	0.930	0.675	0.750	0.750	0.740
Short-circuit current density (ma/sq cm)	0.991	1.470	0.652	0.915	1.070	0.651

II (10 Percent by Weight of I, after 24 hours, and 90 Percent of Fresh Original Mixture)

Time (hours)	0	4	45	72	162
Open-circuit potential (v.)	0.325	0.950	0.725	0.720	0.710
Short-circuit current density (ma/sq cm)	0.171	1.015	0.496	0.745	0.495

III (10 Percent by Weight of II, after 24 hours, and 90 Percent of Fresh Original Mixture)

Time (hours)	0	6	49	72	144
Open-circuit potential (v.)	0.350	0.755	0.635	0.640	0.600
Short-circuit current density (ma/sq cm)	0.062	0.155	0.124	0.170	0.062

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IV (10 Percent by Weight of III, after 24 hours, and 90 Percent of Fresh Original Mixture)

Time (hours)	0	24	32	55	116
Open-circuit potential (v.)	0.425	0.780	0.690	0.660	0.615
Short-circuit current density (ma/sq cm)	---	0.310	0.264	0.171	0.124

V (10 Percent by Weight of IV, after 24 hours, and 90 Percent of Fresh Original Mixture)

Time (hours)	0	1	8	21	31	105
Open-circuit potential (v.)	0.330	0.615	0.975	0.800	0.760	0.425
Short-circuit current density (ma/sq cm)	---	0.559	1.440	1.161	1.082	0.372

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